

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

1. (Currently Amended) An expression vector comprising a polynucleotide which encodes a heterologous fusion protein containing (i) a single signal sequence polypeptide consisting of the signal sequence of the gac gene of *Pseudomonas diminuta* and (ii) a polypeptide of interest[[,]] other than gac gene of *Pseudomonas diminuta*, wherein said signal sequence and said polypeptide of interest are linked ~~in such a way that, so that~~ upon expression of the polynucleotide as a fusion protein in a suitable host cell, the signal sequence is cleaved off the fusion protein and the polypeptide of interest is released into the periplasm of the host cell.
2. (Original) The vector according to claim 1, wherein said vector is a plasmid.
3. (Original) The vector according to claim 1, wherein said vector is a high copy plasmid.
4. (Original) The vector according to claim 1, wherein the polypeptide of interest is interferon alpha 2.
5. (Original) The vector according to claim 4, wherein the interferon alpha 2 is selected from the group consisting of interferon alpha 2A and interferon alpha 2B.
6. (Currently Amended) The vector according to claim 1, wherein said signal sequence of the gac gene of *Pseudomonas diminuta* comprises the amino acid sequence [[()SEQ ID NO: 2[()]]]

MLRVLHRAASALVMATVIGLAPAVAFA.

7. (Previously Presented) The vector according to claim 6, wherein said vector further comprises a second polynucleotide comprising the promoter region and the ribosomal binding site of the gac

gene of *Pseudomonas diminuta*, wherein the second polynucleotide is operatively linked to the polynucleotide encoding the fusion protein comprising the signal sequence and the polypeptide of interest.

8. (Currently Amended) The vector according to claim 7, wherein said second polynucleotide comprising the promoter region and the ribosomal binding site comprises the nucleotide sequence [[()SEQ ID NO: 5[()]]]

5'-ATCCTGGTTCGTACGGCGCCGCCCTACAAGTGGTGATCTAGGGGAACGTTCCGGGGGC  
GTCGCTGCAACGGCGTCTCCGGATCTGGGTGAGAGGGGAAATCC-3'.

9. (Withdrawn-Previously Presented) The vector according to claim 8, wherein said second polynucleotide comprising the promoter region and the ribosomal binding site comprises the nucleotide sequence (SEQ ID NO: 6)

5'-TCTAGACCAACAAACATCTTCAACGTCTACCCGACCAAGATTAGGAGCCGTCGGCC  
GACCTGGGCAATGGGATGTACAGCGGGCTTGCAGCCGTTGGCTTCACCGGCGGAT  
CCTGGTTCGTACGGCGCCCTACAAGTGGTGATCTAGGGGAACGTTCCGGGGCG  
TCGCTGCAACGGCGTCTCCGGATCTGGGTGAGAGGGGAAATCC-3'.

10. (Currently Amended) A prokaryotic host cell containing an expression vector which comprises a polynucleotide which encodes a heterologous fusion protein containing (i) a single signal sequence consisting of the signal sequence of the gac gene of *Pseudomonas diminuta* and (ii) a polypeptide of interest, other than the gac gene of *Pseudomonas diminuta*, wherein said signal sequence and said polypeptide of interest are linked in such a way that, so that upon expression of the polynucleotide as a fusion protein in a suitable host cell, the signal sequence is cleaved off the fusion protein and the polypeptide of interest is released into the periplasm of the host cell, wherein the host cell is stably transformed by the expression vector.

11. (Original) The host cell according to claim 10, wherein said vector is a plasmid.

12. (Original) The host cell according to claim 10, wherein said vector is a high copy plasmid.
13. (Original) The vector according to claim 10, wherein the polypeptide of interest is interferon alpha 2.
14. (Original) The vector according to claim 13, wherein the interferon alpha 2 is selected from the group consisting of interferon alpha 2A and interferon alpha 2B
15. (Currently Amended) The host cell according to claim 10, wherein said signal sequence of the gac gene of *Pseudomonas diminuta* comprises the amino acid sequence [[()SEQ ID NO: 2[()]]

~~MLRVLHRAASALVMATVIGLAPAVAFA.~~

16. (Currently Amended) The host cell according to claim 10, wherein said vector further comprises a second polynucleotide comprising the promoter region and the ribosomal binding site of the gac gene of *Pseudomonas diminuta*, wherein the second polynucleotide is operatively linked to the polynucleotide encoding the fusion protein comprising the signal sequence and the polypeptide of interest, wherein the promoter region promotes expression of both the first and the second polynucleotide.
17. (Currently Amended) The host cell according to claim 16, wherein said second polynucleotide comprising the promoter region and the ribosomal binding site comprises the nucleotide sequence [[()SEQ ID NO: 5[()]]]

~~5' ATCCTGGTTCGTACGGGGGGCCTACAAGTGGTGATCTAGGGGAACGTTCCCCGGGC  
GTCGCTGCAACGGCGTCTCCGGATCTGGGTGAGAGGGGAAATCC 3'.~~

18. (Withdrawn- Previously Presented) The host cell according to claim 16, wherein said second polynucleotide comprising the promoter region and the ribosomal binding site comprises the nucleotide sequence (SEQ ID NO: 6)

5'-TCTAGACCAACAAACATCTTCAACGTCTACCCGACCAAGATTCAAGGAGGCCGTCGGCC  
GACCTGGGCAATGGGATGTACAGCGGGCTTGCACCGCTTCACCGGCAGAT  
CCTGGTTCGTACCGCCGCCTACAAGTGGTGAATCTAGGGAACGTTCCGGGGCG  
TCGCTGCAACGGCGTCTCCGGATCTGGGTGAGAGGGGAAATCC-3'

19. (Original) The host cell according to claim 10, wherein said host cell is an *E. coli* cell.
20. (Currently Amended) A process for production of a polypeptide of interest, comprising:
  - (i) providing a prokaryotic host cell transformed with an expression vector which is compatible with the host cell, said vector comprising a polynucleotide which encodes a heterologous fusion protein which comprises (a) a single signal sequence consisting of the signal sequence of the gac gene of *Pseudomonas diminuta* and (b) a polypeptide of interest, other than the gac gene of *Pseudomonas diminuta*, wherein said signal sequence and said polypeptide of interest are linked in such a way that, so that upon expression of the polynucleotide as a fusion protein in a suitable host cell, the signal sequence is cleaved off the fusion protein and the polypeptide of interest is released into the periplasm of the host cell;
  - (ii) culturing the prokaryotic host cell under conditions which cause expression of the polynucleotide as a fusion protein, whereby upon formation of the fusion protein the signal sequence is cleaved off the fusion protein and the polypeptide of interest is released into the periplasm of the host cell; and
  - (iii) isolating the polypeptide of interest from the host cell.
21. (Canceled)
22. (Original) The process according to claim 20, wherein said vector is a plasmid.
23. (Original) The process according to claim 20, wherein said vector is a high copy plasmid.
24. (Original) The vector according to claim 20, wherein the polypeptide of interest is interferon alpha 2.

25. (Original) The vector according to claim 24, wherein the interferon alpha 2 is selected from the group consisting of interferon alpha 2A and interferon alpha 2B.

26. (Currently Amended) The process according to claim 20, wherein said signal sequence of the gac gene of *Pseudomonas diminuta* comprises the amino acid sequence [[()SEQ ID NO: 2[()]]

~~MLRVLHRAASALVMATVIGLAPAVAFA.~~

27. (Currently Amended) The process according to claim 20, wherein said vector further comprises a second polynucleotide comprising the promoter region and the ribosomal binding site of the gac gene of *Pseudomonas diminuta*, wherein the second polynucleotide is operatively linked to the polynucleotide encoding the fusion protein comprising the signal sequence and the polypeptide of interest, wherein the promoter region promotes expression of both the first and the second polynucleotide.

28. (Currently Amended) The process according to claim 27, wherein said second polynucleotide comprising the promoter region and the ribosomal binding site comprises the nucleotide sequence [[()SEQ ID NO: 5[()]]]

5'-ATCCTGGTTCGTACCGCGCCGCTACAAGTGGTGATCTAGGGGAACGTTCCGGGGGC  
GTCGCTGCAACGGCGTCTCCGGATCTGGGTGAGAGGGGAAATCC-3'.

29. (Withdrawn- Previously Presented) The process according to claim 27, wherein said second polynucleotide comprising the promoter region and the ribosomal binding site comprises the nucleotide sequence (SEQ ID NO: 6)

5'-TCTAGACCAACAAACATCTTCAACGTCTACCCGACCAAGATTAGGAGCCGTCGGCC  
GACCTGGGCAATGGGATGTACAGCGGGCTTGCAGCCGTTGGCTTCACCGGCAGAT  
CCTGGTTCGTACCGCGCCGCTACAAGTGGTGATCTAGGGGAACGTTCCGGGGCG  
TCGCTGCAACGGCGTCTCCGGATCTGGGTGAGAGGGGAAATCC-3'

30. (Original) The process according to claim 20, wherein said host cell is an *E. coli* cell.
31. (Original) The process according to claim 20, said culturing being performed as a multi-stage fermentation process comprising a shake-flask step, optionally a pre-culture step, and a main-culture step.
32. (Original) The process according to claim 31, wherein said culturing of the prokaryotic host cell in the main culture step is performed in a culture medium comprising a substrate for more than about 90% of the cultivation time at a substrate concentration lower than the saturation constant of the substrate, accompanied by high levels of dissolved oxygen concentration, and further accompanied by a steadily decreasing specific growth rate of the bacterial host cells, the process being performed at a temperature which is lower than the optimum temperature for growth of the host cell.
33. (Original) The process according to claim 32, wherein the concentration of dissolved oxygen in the main culture step is from about 40 % up to about 100% of saturation.
34. (Original) The process according to claim 32, wherein the steadily decreasing growth rate in the main culture step is from about  $2 \text{ h}^{-1}$  to about  $0.001 \text{ h}^{-1}$ .
35. (Original) The process according to claim 32, wherein the temperature in the main culture step is between about  $22^\circ\text{C}$  and about  $35^\circ\text{C}$ .
36. (Original) The process according to claim 35, wherein the temperature in the main culture step is between about  $25^\circ\text{C}$  and about  $31^\circ\text{C}$ .
37. (Original) The process according to claim 36, wherein the temperature in the main culture step is about  $28^\circ\text{C}$ .
38. (Original) The process according to claim 32, wherein said process is performed at a pH value in the range of about 6.7 to about 7.3 in the pre-culture step and/or the main-culture step.

39. (Original) The process as claimed in claim 32, wherein the substrate is a carbohydrate or glycerol.

40. (Original) The process according to claim 39, wherein the carbohydrate is glucose.

41. (Original) The process according to claim 32, wherein the host cell is an *E. coli* cell.

42. (Currently Amended) A prokaryotic host cell transformed with an expression vector which is compatible with the host cell, said vector comprising:

a) a first polynucleotide encoding a fusion protein which comprises i) the a single signal sequence consisting of the signal sequence of the a gac gene of *Pseudomonas diminuta* and ii) a polypeptide of interest selected from the group consisting of human interferon alpha 2A and human interferon alpha 2B, wherein said signal sequence and said polypeptide of interest are linked in such a way that, so that upon expression of the first polynucleotide as a fusion protein in a suitable host cell, the signal sequence is cleaved off the fusion protein and the polypeptide of interest is released into the periplasm of the host cell, wherein the host cell is an *E. coli* cell; and

b) a second polynucleotide comprising the a promoter region and the a ribosomal binding site of the gac gene of *Pseudomonas diminuta*, wherein the second polynucleotide is operatively linked to the first polynucleotide encoding the fusion protein comprising the signal sequence and the polypeptide of interest, wherein the promoter region promotes expression of both the first and the second polynucleotide.

43. (Currently Amended) A process for production of a polypeptide of interest, comprising:

(i) providing a prokaryotic host cell transformed with an expression vector which is compatible with the host cell, said vector comprising:

—a) a polynucleotide encoding a fusion protein which comprises i) a single the signal sequence, wherein the signal sequence consists of the signal sequence of gac gene of *Pseudomonas diminuta*; the a promoter region[[,]] of gac gene of *Pseudomonas diminuta*; and the a ribosomal binding site of the gac gene of *Pseudomonas diminuta*; and ii) a polypeptide of

interest selected from the group consisting of human interferon alpha 2A and human interferon alpha 2B, wherein said signal sequence and said polypeptide of interest are linked in such a way that, so that upon expression of the first polynucleotide as a fusion protein in a suitable host cell, the signal sequence, promoter region and ribosomal binding site are is cleaved off the fusion protein, and the polypeptide of interest is released into the periplasm of the host cell, wherein the promoter region promotes expression of both the first and the second polynucleotide, and wherein the host cell is an E. coli cell; and

- (ii) culturing the prokaryotic host cell under conditions which cause expression of the first polynucleotide whereby upon formation of the fusion protein the signal sequence is cleaved off the fusion protein and the polypeptide of interest is released into the periplasm of the host cell; and
- (iii) isolating the polypeptide of interest from the host cell.